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Rationale: **Abdominal aortic aneurysm (AAA) is a complex disease with both genetic and environmental risk factors. Together, 6 previously identified risk loci only explain a small proportion of the heritability of AAA.**

Objective: **To identify additional AAA risk loci using data from all available genome-wide association studies.**

Methods and Results: **Through a meta-analysis of 6 genome-wide association study data sets and a validation study totaling 10 204 cases and 107 766 controls, we identified 4 new AAA risk loci: 1q32.3 (*SMYD2*), 13q12.11 (*LINC00540*), 20q13.12 (near *PCIF1/MMP9/ZNF335*), and 21q22.2 (*ERG*). In various database searches, we observed no new associations between the lead AAA single nucleotide polymorphisms and coronary artery disease, blood pressure, lipids, or diabetes mellitus. Network analyses identified *ERG*, *IL6R*, and *LDLR* as modifiers of *MMP9*, with a direct interaction between *ERG* and *MMP9*.**

Conclusions: **The 4 new risk loci for AAA seem to be specific for AAA compared with other cardiovascular diseases and related traits suggesting that traditional cardiovascular risk factor management may only have limited value in preventing the progression of aneurysmal disease.**

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Key Words: aortic aneurysm, abdominal ■ computational biology ■ genetics ■ genome-wide association study ■ matrix metalloproteinases ■ meta-analysis

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For the author affiliations, please see the Appendix.

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Novelty and Significance

What Is Known?

- Abdominal aortic aneurysm (AAA) has a prevalence of ≈1.5% in men aged >65 years.
- Positive family history of AAA is a strong risk factor for AAA; however, only 6 robust and independently validated AAA genetic loci have been identified to date.

What New Information Does This Article Contribute?

- Four novel genetic loci associated with AAA were identified.
- Pathway analysis highlighted the potential importance of lipoprotein metabolism, inflammation, and matrix metalloproteinases in AAA pathobiology.
- Potentially novel mechanisms, involving genes such as *ERG, PLTP*, and *FGF9*, were implicated.

AAA is a significant health burden, particularly among elderly males. It has a strong heritable component; however, previously identified risk loci explain only a small proportion of this effect. No current effective medical therapies that slow AAA growth exist, highlighting the need to better understand factors influencing pathogenesis and disease progression. This study is the first meta-analysis of genome-wide association studies for AAA (10 204 cases). Four novel loci were identified and 5 of the 6 previous AAA genetic associations were confirmed. The new loci showed no significant associations with other arterial disease phenotypes, potentially suggesting associations more specific to AAA than known loci (such as *CDKN2BAS1*, *SORT1*, and *LDLR*). Associations were consistent with known AAA pathobiology, implicating lipoprotein metabolism, inflammation, and matrix metalloproteinases but also identified potentially novel mechanisms relating to genes such as *ERG* and *FGF9*. This study has identified novel, potentially disease-specific, genetic associations with AAA. Further functional studies, investigating the translational potential of these observations, will be required.

Nonstandard Abbreviations and Acronyms

AAA	abdominal aortic aneurysm
CAD	coronary artery disease
eQTL	expression quantitative trait locus
GWAS	genome-wide association study
IL	interleukin
IPA	ingenuity pathway analysis
LDLR	low-density lipoprotein receptor
LRP1	low-density lipoprotein receptor related protein 1
SMYD2	SET and MYND domain containing 2 (SET domain-containing proteins, such as catalyze lysine methylation)
SNP	single nucleotide polymorphism
TNF	tumor necrosis factor

Abdominal aortic aneurysms (AAAs; MIM100070) are a significant cause of mortality and morbidity in the Western world. Although much less common than ischemic heart disease or stroke, AAA is responsible for ≈11 000 deaths/y in the United States, with no clinical treatment other than expensive, high-risk surgery.[1] The US Preventative Services taskforce recommends AAA screening by ultrasound for all men aged 65 to 75 years who have ever smoked.[2] The UK NHS AAA Screening Program screens all men at the age of 65 years irrespective of smoking history yielding a prevalence of AAA (>29 mm) of 1.2%.[3]

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AAA is an enigmatic complex disease. Although sharing risk factors for, and often coexisting with atherosclerosis, AAA can be considered to be a distinct entity from atherosclerosis. Smoking, a positive family history of AAA, and male sex have been consistently identified as the strongest risk factors for AAA. There is uncertainty over the influence of other traditional cardiovascular risk markers such as hypertension and hyperlipidemia. Furthermore, diabetes mellitus has been found to be negatively associated with AAA and is strongly protective against disease progression (AAA growth).[1]

Heritability of AAA is >0.7,[4] and individuals with a first-degree relative with AAA have a 2-fold higher risk of developing an AAA.[5] Genome-wide association studies (GWAS) have identified 3 AAA risk loci on chromosomes 9 (*DAB2IP*[6] [DAB2 interacting protein]), 12 (*LRP1*[7] [low-density lipoprotein receptor related protein 1]), and 19 (*LDLR*[8] [low-density lipoprotein receptor]). Further AAA risk loci on chromosomes 1 (*SORT1*[9] [sortilin 1] and *IL6R*[10] [interleukin 6 receptor]) and 9 (*CDKN2BAS1/ANRIL*[11] [also known as CDKN2B-AS1, CDKN2B antisense RNA 1]) were identified by candidate gene/locus approaches. Together, these explain only a small proportion of the heritability of AAA.

Overall, the high heritability estimates for AAA and the small number of loci identified suggest that there are further risk loci yet to be found. In the current study, we performed a meta-analysis of 6 available GWAS data sets for AAA on 4972 cases and 99 858 controls and confirmed the findings within validation data sets of 5232 cases and 7908 controls. This resulted in identification of 4 novel validated loci for AAA. We followed up positive results with extensive bioinformatics analyses and used data available from various databases to elucidate the potential biological significance of our findings to the pathobiology of AAA.

Methods

Detailed Methods are available in the Online Data Supplement.

Expanded Aneurysm Consortium

All known studies with AAA genome-wide genotyping (Online Methods; Online Table I) were invited to join the International Aneurysm Consortium. Additional samples (Online Methods; Online Table II) were used for the validation study. All AAA cases had an infrarenal aortic diameter of >30 mm. AAAs secondary to connective tissue diseases were excluded. The use of the samples in each study cohort was approved by local Ethics Committees or Institutional Review Boards.

Meta-Analysis

The discovery phase of the meta-GWAS was conducted using the METAL (a tool for meta-analysis of genome-wide association scans) software package[12] on the 6 cohorts detailed in Online Table I, comprising 4972 AAA cases and 99 858 controls. An effective sample number (N_{eff}) weighted analysis[12] was conducted because of case/control asymmetry within some of the contributing cohorts. Quality control included assessments for population stratification in each data set and adjustment was performed if necessary. The analysis of each contributing GWAS had been performed independently, and there was therefore no uniform analysis plan across all data sets. The individual GWAS data sets from Iceland and the Netherlands were adjusted for genomic inflation before inclusion in the meta-analysis. The overall meta-analysis was then adjusted for genomic inflation (λ; Online Table I; Online Figure I). An initial (λ-adjusted) discovery threshold of $P<5\times10^{-6}$ was used to identify single nucleotide polymorphisms (SNPs) for subsequent validation genotyping. SNPs with high heterogeneity ($P_{het}<0.005$ or $I^2>70\%$) were not taken forward for validation.

The lead SNPs [or their proxies in high linkage disequilibrium], identified in the discovery analyses, were then genotyped in a further 8 independent cohorts with 5,232 cases and 7,908 controls (Online Table II). Allele association analysis of each individual validation study cohort was carried out using the SHEsis (software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci) web-based software package.[13] A combined (discovery-validation) fixed effect meta-analysis was performed using a Maentel–Haenzel method with the genome-wide *P*-value significance threshold being set at 5×10^{-8}. Random-effects (Han-Eskin method[14]) meta-analysis was also performed to determine whether any results were sensitive to between-study heterogeneity.

SNP Lookup in GWAS for Other Traits Associated With AAA

GWAS data sets for other traits were searched for associations with the AAA-associated SNPs to determine whether the associations were unique to AAA or related to generalized cardiovascular disease. Results were obtained from meta-analyses of multiple primary GWAS data sets for each trait. Summary data for each AAA associated SNP (*P* value and effect size) were extracted. *P* values $<5\times10^{-8}$ were considered to be significant. Results were available for type 2 diabetes mellitus[15] (DIAGRAM [a consortium called DIAbetes Genetics Replication And Meta-analysis] consortium; http://www.diagram-consortium.org/index.html), coronary artery disease (CAD; CARDIoGRAM consortium (a consortium called Coronary ARtery DIsease Genome wide Replication and Meta-analysis)[16]; www.CARDIOGRAMPLUSC4D.ORG), lipids (the Global Lipids Genetics Consortium[17]; http://csg.sph.umich.edu/abecasis/public/lipids2013), and blood pressure (the International Consortium for Blood Pressure[18]; http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000585.v1.p1).

Search for Other Associated Traits and Diseases Using GWAS Databases

The Phenotype-Genotype Integrator[19] (http://www.ncbi.nlm.nih.gov/gap/phegeni#GenomeView), the GWAS catalog (http://www.gwascentral.org/index), and the NHLBI GRASP (The Genome-wide Repository of Associations between SNPs and Phenotypes) catalog (GRASP v2.0; http://grasp.nhlbi.nih.gov/Overview.aspx)[20] were searched for diseases and traits associated with the lead SNPs at the AAA loci.

Phenome-Wide Association Study Analysis

We performed a phenome-wide association study (PheWAS)[21,22] exploring associations between the 9 AAA-associated SNPs and an extensive group of diagnoses to identify novel associations and uncover potential pleiotropy. For the PheWAS, we used data from the eMERGE (electronic Medical Records and Genomics) Network[23] with a total of 27 077 unrelated patients of European ancestry aged >19 years. We divided these samples into 2 data sets by proportional sampling based on eMERGE site, sex, and genotyping platform (13 559 and 13 518 individuals in sets 1 and 2, respectively). We calculated associations between the 9 AAA-associated SNPs and case or control status based on the extensive set of 9th edition of the *International Statistical Classification of Diseases* and Related Health Problems diagnoses (2408 and 2385 in sets 1 and 2, respectively) where for a specific diagnosis, individuals with the diagnosis are considered cases. Associations were adjusted for sex, site, genotyping platform, and the first 3 principal components to account for global ancestry.

Annotation of AAA Associated SNPs Using the University of California Santa Cruz Genome Browser, Pupasuite, and GWAS3D

Confirmed AAA-associated loci were manually annotated using the University of California Santa Cruz Genome Browser (http://genome.ucsc.edu/cgi-bin/hgGateway) on the hg19 human genome assembly. For the Pupasuite analyses SNPs in linkage disequilibrium ($r^2>0.5$) and with lead SNPs at the novel AAA risk loci identified were extracted from the 1000 Genomes data and then entered into Pupasuite v3.1.[24] In addition, all known (novel and previously identified) AAA-associated SNPs were entered into the GWAS3D (bioinformatics tool detecting human regulatory variants by integrative analysis of genome-wide associations, chromosome interactions, and histone modifications)[25] web-portal (http://jjwanglab.org/gwas3d) to identify functional SNPs.

Bioinformatic Identification of Candidate AAA Genes and Pathways Using DEPICT (Data-Driven Expression-Prioritized Integration for Complex Traits)

An integrated gene function analysis was performed using the DEPICT tool (version 1.1).[26] Two separate runs were performed using either all independent SNPs with discovery meta-GWAS $P<5\times10^{-6}$ or just those 9 SNPs that reached $P<5\times10^{-8}$ in the combined analysis. Both nominal *P* values and false discovery rates were calculated.

Experimental Evidence for Functional Variants at AAA Loci

SNPs at loci confirmed to be associated with AAA were examined for functional effects using multiple methods (Online Methods). (1) To search for evidence of functional effects of SNPs at AAA associated loci 2 expression quantitative trait locus (eQTL) data sets based on publically available data, and a broad range of tissues with relatively large sample sizes were examined. First, index and proxy SNPs were queried in a collected database of published expressed SNP results. The collected expressed SNP results met criteria for statistical thresholds for association with gene transcript levels as described in the original publications. Second, additional eQTL data were integrated from online sources including ScanDB (SNP and CNV Annotation Database), the Broad Institute The Genotype-Tissue Expression browser, and the Pritchard Laboratory (eqtl.uchicago.edu). (2) To search for vascular tissue-specific effects, eQTL data were also obtained from the ASAP (Advanced Study of Aortic Pathology) data set[27] and RNA-seq (whole-genome RNA-sequence generated by high-throughput methods) data were from the Stockholm-Tartu Atherosclerosis Reverse Network Engineering Task (STARNET) database[28] (http://www.mountsinai.org/profiles/johan-bjorkegren). (3) Because some genes at AAA loci were associated with monocyte function and AAA is known to be an inflammatory disease,[29] data from an eQTL analysis of peripheral blood monocytes were obtained from the Cardiogenics Consortium (http://www.cardiogramplusc4d.org/). (4) Finally to search for effects in AAA tissue specifically, mRNA expression profiles of all the GWAS3D predicted distal targets, as well as SNP proximity implicated genes, were examined using a previously published genome-wide expression data set on human aorta (GSE57691),[30]

from which 49 AAA samples were compared with 10 organ donor control aortic samples. Transcription factor (TF) binding data were also obtained from a previous study,[31] which described chromatin-immunoprecipitation (ChIP)-chip for TFs ELF1, ETS2, RUNX1, and STAT5 using human aortic tissue in AAAs and healthy control aorta.

Network Analysis

We investigated whether most of the loci could be connected into a single network through intermediate nodes and interactions. A network integrating most of the loci would suggest mechanisms by which the loci could act in concert, whether synergistically or antagonistically, to affect the phenotype. The network(s) would also provide hypotheses for future investigation. Using the genes harboring AAA-associated SNPs as a starting set, we analyzed potential interactions between the proteins and known intermediates (proteins, noncoding RNA, and metabolites) using 2 independent analysis tools, Ingenuity Pathway Analysis (IPA) tool version 9.0 (Qiagen's Ingenuity Systems, Redwood City, CA; www.ingenuity.com) and Consensus PathDB (http://cpdb.molgen.mpg.de/CPDB).[32,33] The analyzed gene set had 14 genes because 2 of the 9 AAA loci included clusters of 3 genes and tumor necrosis factor (TNF) was added because of the recent literature demonstrating the strong effect of SMYD2 (SET and MYND domain containing 2 [SET domain-containing proteins, such as catalyze lysine methylation]) on interleukin-6 (IL6) and TNF production[34,35] (see Online Table XIV for SNP annotations and Online Methods).

Results

Meta-Analysis of 6 GWAS Data sets for AAA Followed by a Validation Study Reveals 4 New AAA Susceptibility Loci

The meta-analysis of 6 GWAS data sets (4972 AAA cases; 99 858 controls; Online Table I) revealed 19 loci of interest ($P<1\times10^{-6}$, Online Tables III and IV; Figure 1). Lead SNPs from these loci, including the 6 AAA risk loci reported previously, were analyzed in a validation study of 5232 AAA cases and 7908 controls (Online Tables II, V, VI, and VII). Four new loci were independently significant ($P<0.05$) in the validation cohort, had a direction of effect consistent with the discovery cohort and when combined with the discovery cohort had a P value that surpassed a genome-wide significance (5×10^{-8}): 1q32.3 (*SMYD2*), 13q12.11 (*LINC00540* [long intergenic nonprotein coding RNA 540]), 20q13.12 (near *PCIF1* [C-terminal inhibiting factor 1 of a protein called pancreatic and duodenal homeobox 1]/*MMP9* [matrix metalloproteinase 9]/*ZNF335* [zinc finger protein 335]), and 21q22.2 (*ERG* [v-ets avian erythroblastosis virus E26 oncogene homolog]; Table 1; Online Tables V, VI, and VII; Figure 2). All previously reported associations with AAA were confirmed at genome-wide significance (Table 1; Online Table VII; Online Figure II) with the exception of 12q13.3 (*LRP1*), where the lead SNP identified in this meta-analysis and tested in our validation study only demonstrated a borderline association with AAA in the combined analysis ($P=6.4\times10^{-7}$). There was evidence of significant heterogeneity in the results observed for rs1795061 (near *SMYD2*) and rs2836411 (*ERG*) (Online Table VII). A random-effects model sensitivity analysis (HanEskin[14] method) demonstrated minimal effect on the results for these 2 loci (Online Table VIII). The lead SNPs at 2 loci that were both below the threshold for genome-wide significance under the fixed-effects model (rs6516091, 20p12.3, near *FERMT1* and rs5954362, Xq27.2, *SPANXA1*) were significant

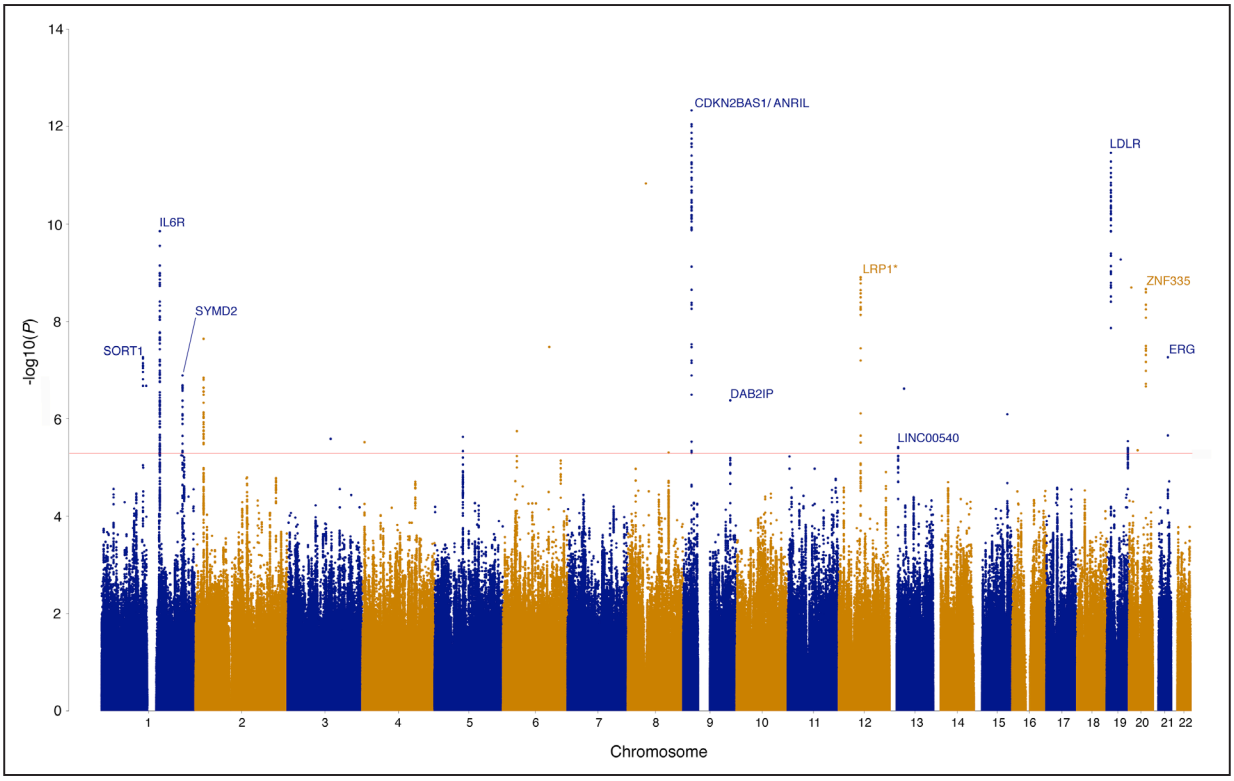

Figure 1. Whole-genome association plot for the primary meta-analysis of genome-wide association studies of abdominal aortic aneurysm (AAA). Data represent a meta-analysis of 4972 AAA cases and 99 858 controls. The horizontal line indicates the P value threshold of 5×10^{-6} used to select loci for validation studies. The 9 subsequently validated AAA loci are indicated along with the previously identified *LRP1* locus, which fell to $P=6.4\times10^{-7}$ in the combined discovery/ validation analysis (Online Tables III and IV).

Table 1. List of AAA Associated Loci Surpassing a Genome-Wide Significance Threshold After Combining GWAS data (4972 cases and 99 858 controls) and Validation Data (5232 cases and 7908 controls)

							Discovery Phase			Validation Phase			Combined			
SNP	Chr	Position	Nearest Gene(s)	Min_All*	Maj_All*	MAF	OR	*P* Value	I^2	OR	*P* Value	I^2	OR	95% CI	*P* Value	I^2
Previously reported AAA risk loci																
rs602633	1	109821511	*PSRC1-CELSR2-SORT1*	T	G*	0.199	0.845	3.12×10^{-08}	29.4	0.920	9.83×10^{-3}	55.7	0.879	0.842–0.918	6.58×10^{-9}	**54.5**
rs4129267	1	154426264	*IL6R*	T	C*	0.370	0.854	1.74×10^{-10}	0	0.904	1.81×10^{-4}	17.2	0.876	0.846–0.908	4.76×10^{-13}	**0.0**
rs10757274	9	22096055	*CDKN2BAS1/ ANRIL*	A	G*	0.462	0.832	2.71×10^{-13}	10.0	0.774	1.02×10^{-21}	64.2	0.806	0.778–0.834	1.54×10^{-33}	**55.6**
rs10985349	9	124425243	*DAB2IP*	T*	C	0.195	1.185	2.01×10^{-7}	18.1	1.155	2.30×10^{-5}	3.9	1.171	1.118–1.226	2.40×10^{-11}	**9.2**
rs6511720	19	11202306	*LDLR*	T	G*	0.096	0.743	8.60×10^{-13}	0	0.868	6.02×10^{-4}	68.2	0.804	0.759–0.851	7.90×10^{-14}	**61.0**
Novel AAA risk loci																
rs1795061	1	214409280	*SMYD2*	T*	C	0.337	1.154	$3.26\times10–^{8}$	47.9	1.105	3.49×10^{-4}	70.3	1.131	1.090–1.174	8.80×10^{-11}	**61.9**
rs9316871	13	22861921	*LINC00540*	A	G*	0.201	0.864	1.23×10^{-6}	33.2	0.883	8.28×10^{-5}	0.0	0.873	0.837–0.911	4.75×10^{-10}	**0.0**
rs3827066	20	44586023	*PCIF1-ZNF335-MMP9*	T*	C	0.179	1.232	1.88×10^{-10}	0	1.213	2.00×10^{-8}	16.5	1.223	1.168–1.281	2.13×10^{-17}	**0.0**
rs2836411	21	39819830	*ERG*	T*	C	0.369	1.149	2.51×10^{-8}	30.1	1.072	1.13×10^{-2}	28.3	1.113	1.074–1.154	5.80×10^{-9}	**42.2**

For all loci shown the direction of effect was consistent across all studies in the discovery phase. Full details are shown in Online Tables III, IV, V, VI, and VII. Results shown for the discovery, validation and combined analyses are all Maentel–Haenzel fixed effect meta-analysis method. AAA indicates abdominal aortic aneurysm; CI, confidence interval; MAF, minor allele frequency; and OR, odds ratio.

*Effect allele.

in the random-effects model. However, because both demonstrated extreme heterogeneity ($I^2 \geq 0.7$), we did not consider these to be newly identified loci for AAA and these were excluded from further analysis.

New AAA Loci Seem to be Specific for AAA

To assess whether the loci identified in our meta-analysis were specific to AAA or were also associated with diseases or risk factors known to be associated with AAA, we looked up results from GWAS of CAD,[6] hypertension,[18] and lipid traits.[17] We also obtained results for diabetes mellitus[15] to determine whether there was a reverse effect at these loci because diabetes mellitus is a negative risk factor for AAA and negatively influences AAA growth.[1] Other than the known associations at 1p13.3 (*SORT1*), 9p21 (*CDKN2BAS1/ANRIL)* with CAD, 1p13.3 (*SORT1*) with high-density lipoprotein/LDL, and 19p13.2 (*LDLR*) with LDL, we observed no new associations between the lead SNPs at any of the AAA risk loci we had identified and these traits (Figure 3; Online Table IX). In particular, no association was observed between diabetes mellitus and these SNPs. Literature searching revealed an association between rs4845625 at 1q21.3 (*IL6R*) and CAD, but this was not in high linkage disequilibrium with the lead SNP genotyped in our study at this locus (R^2=0.54).[36]

We also searched GWAS Central (database proving integrative visualization of and access to GWAS data) and Phenotype-Genotype Integrator and performed a GRASP[37] analysis for any associations of the lead AAA SNPs with traits other than those listed above. We identified additional genome-wide significant associations between 1q21.3/*IL6R* (rs4129267) and C-reactive protein/asthma, and nominal associations between 1p13.3/*SORT1* (rs602633), 21q22.2/*ERG* (rs2836411), and 19p13.2/*LDLR* (rs6511720) and height (Online Tables X, XI, and XII), a potential risk factor for AAA.[38]

We also performed a PheWAS[21,22] in the eMERGE data sets exploring the association between the 9 AAA-associated SNPs and an extensive group of diagnoses to identify novel associations and uncover potential pleiotropy. We considered identification of previously known associations, such as rs602633 associated with hyperglyceridemia and rs10757274 associated with CAD, to be indications that the PheWAS approach was robust. The PheWAS results demonstrated the known associations with CAD and lipid levels but did not identify any novel disease associations (Online Table XIII).

Annotation of SNPs at AAA Loci

Annotation did not identify any nonsynonymous variants in high linkage disequilibrium (R^2>0.5) with the lead SNPs at the AAA risk loci (Online Tables XIV and XV). Based on GWAS3D analysis, all 9 lead SNPs were associated with TF-binding site affinity variants (Online Tables XVI and XVII). Eight SNPs had potential long-range interactions with distal genomic regions (Figure 4). GWAS3D analysis also provided potential mechanistic insight for intergenic AAA variants such as rs9316871 (13q12.11) that had significant predicted regulatory variant interaction with *FGF9* (fibroblast growth factor 9; 13q12.11). In addition, although the AAA association with rs599839 (1p13.3) showed strong long-range chromatin interaction with SORT1 (as previously reported specifically in AAA[9]), it also had predicted distal interactions with other genes including *BCAR3* (breast cancer antiestrogen resistance 3; 1p22.1) and *NOTCH2* (notch 2 member of type 1 transmembrane protein family; 1p12-p11).

DEPICT Gene Pathway Prediction

DEPICT identified 633 and 482 gene enrichment sets with nominal *P*<0.05 using the discovery meta-GWAS SNP set

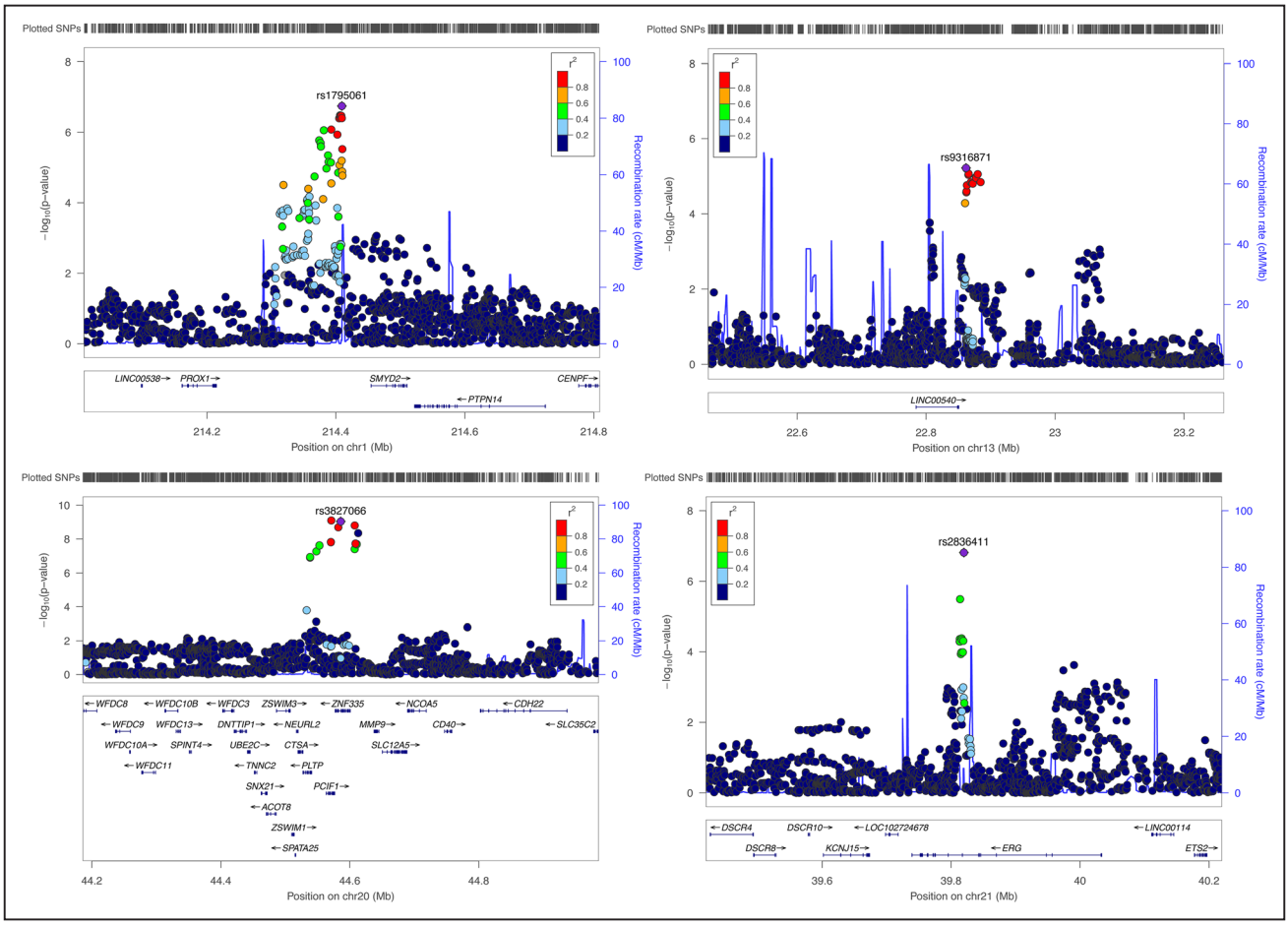

Figure 2. Regional association plots for 4 new abdominal aortic aneurysm (AAA) genome-wide significant loci at 1q32.3, 13q12.11, 20q13.12, and 21q22.2. New AAA genome-wide significant loci at 1q32.3 (near *SMYD2*), 13q12.11 (*LINC00540*), 20q13.12 (near *MMP9/ZNF335*), and 21q22.2 (*ERG*). $-\log_{10}$ (P_{fixed}) values for single nucleotide polymorphisms (SNPs) from the AAA discovery meta-analysis of 4972 cases and 99 858 controls were plotted against their genomic positions using LocusZoom (1000Genomes, EUR, November 2014). The peak SNP in each region is labeled (purple diamond), whereas the color indicates LD (r^2) with the peak.

($P<5\times10^{-6}$) and top 9 SNPs from the combined analysis, respectively. Only one of the gene sets (decreased long bone epiphyseal plate size) had a false discovery rate of <0.2. Gene set descriptions included multiple functional classes relevant to vascular biology, ie, transforming growth factor-β regulation, lipoprotein metabolism, inflammation-induced extracellular matrix remodeling (regulatory factor X1), vascular smooth muscle cell function, vascular injury including hemorrhage, immune cell function (particularly T and B cells), acute phase response including IL6 secretion, apoptosis, hyperglycemia and the phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha, c-Jun N-terminal kinase, and mitogen-activated kinase-like protein cascades. In addition, there were multiple gene sets associated with long bone size and epiphyseal plate formation (Table 2; Online Table XVIII and Online Data File).

Functional Effects of SNPs at AAA Loci

The lookup of SNPs at AAA loci in studies of functional effects included multitissue eQTL studies, vascular/monocyte-specific eQTL, and AAA-specific studies (mRNA expression and chromatin-immunoprecipitation-chip). These analyses revealed several potential functional associations (Online Tables XI, XX, XXI, and XXII; Online Figure III).[27,39] Of most relevance to AAA, eQTLs were observed for rs3827066 (20q13.3) and *PLTP* (phospholipid transfer protein) expression in aortic tissue and for rs4129267 (1q21.3) and *IL6R* expression in mammary artery. RNA-Seq data also demonstrated independent eQTLs in mammary artery for 2 of the novel AAA associations we have identified: rs2836411 and *ERG* expression and rs9316871 and *FGF9* expression. All eQTLs, with the exception of rs9316871 and *FGF9* were also seen in tissues other than arterial samples.

Several GWAS3D-predicted distal interacting genes had significantly different mRNA expression between AAA and control samples (Table 3; Online Table XXIII and Figure IV).[30] For example, *BCAR3* had decreased mRNA expression in AAA tissue (as did *SORT1* itself). In addition, although the closest gene to rs9316871, a long intergenic noncoding RNA (*LINC00540*), was not part of the mRNA data set, the predicted distal target *FGF9* had significantly increased mRNA expression in AAA tissue (Online Table XXIII).

Chromatin-immunoprecipitation-chip data from human AAA tissue[31] revealed TF-binding sites in 5 genes (*SMYD2, SORT1, CDKN2BAS1/ANRIL*, *ERG*, and *DAB2IP*), which harbor AAA risk loci, but none of these binding sites included

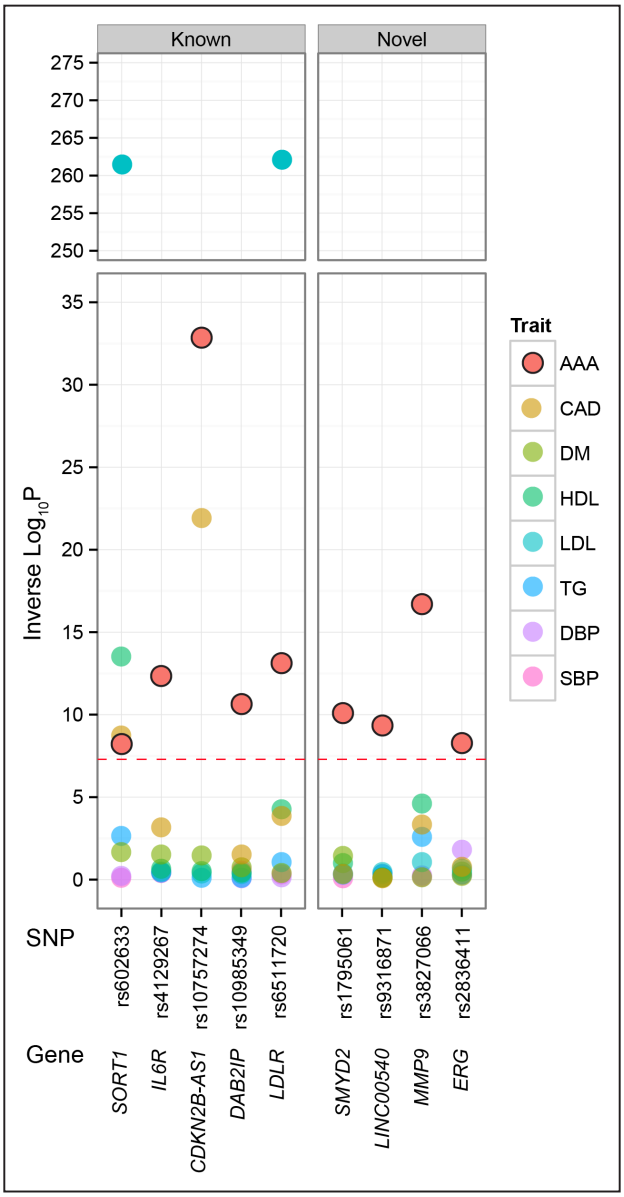

Figure 3. Association between the lead single nucleotide polymorphisms (SNP) at the abdominal aortic aneurysm risk loci and association *P* values for other cardiovascular risk factors/traits (Online Table IX). CAD indicates coronary artery disease; DBP, diastolic blood pressure; DM, diabetes mellitus; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; and TG, triglyceride.

the lead SNP tested for association with AAA (Online Table XXIV).

Network Analysis Reveals a Central Role for Matrix Metalloproteinase 9

Network analysis using both IPA and Consensus PathDB demonstrated similar results (Online Figures V and VI). Both analyses revealed a central role for MMP9 in AAA, with IPA identifying direct interactions (physical contact between 2 molecules such as binding or phosphorylation) between ERG, IL6R and LDLR, and MMP9, and Consensus PathDB identifying a direct interaction between ERG and MMP9 with secondary interactions (interactions without physical contact, such as signaling events) between both SMYD2 and LDLR, and MMP9. On removing TNF from the analysis (which had been added based on the strong effect of SMYD2 on IL6 and TNF production[34,35]), the genes at AAA loci each remained in independent subnetworks. Inclusion of transforming growth factor-B1, implicated in thoracic aneurysms and Marfan syndrome, instead of TNF failed to coalesce the subnetworks. The long noncoding RNA ANRIL (*CDKN2BAS1*), our strongest hit in the genome (Figure 1), has been reported in numerous studies as a GWAS hotspot and a candidate gene for CAD, intracranial aneurysms, and diverse cardiometabolic disorders[40]; however, this was not represented in either the IPA or Consensus PathDB networks.

Discussion

The present study is the largest genetic association study of AAA performed to date, utilizing 6 GWAS data sets for AAA with a total of 4972 cases and 99 858 controls. Furthermore, we used an independent validation set of 5232 AAA cases and 7908 controls and then performed a pooled analysis of all 10 204 cases and 107 766 controls. We confirmed the association of 5 previously reported loci and identified 4 new loci associated with AAA at genome-wide levels of significance. In contrast to previously identified loci, lead SNPs at the newly identified loci did not demonstrate evidence of cross-phenotype association with other cardiometabolic phenotypes. In summary, the genetic evidence to date mirrors that seen in the epidemiological literature where it is clear that AAA and other forms of cardiovascular diseases are seen as distinct but overlapping phenotypes.

Previous genetic discoveries in AAA have pointed to inflammation and immune function (*IL6R* and *CDKN2BAS1/ANRIL*) and low-density lipoprotein metabolism (*SORT1* and *LDLR*) as important mediators of AAA development. The genes at the novel AAA loci identified here are relevant to aneurysm biology, but their precise roles require further investigation. *MMP9* is within the 20q13.12 locus and matrix degradation via MMP9 is known to play a key role in the development of AAA, evidenced by the observation of high levels of MMP9 in end-stage disease specimens.[41] This is also an important finding given the development of novel pharmacotherapies that target inflammation and matrix degradation pathways such as tofacitinib (a novel Janus kinase inhibitor). Although it is tempting to assume that MMP9 is the causal association at this locus, there are, however, other candidate genes at this locus. Examination of the region and the association pattern with AAA (Figure 2) shows that the strongest signals are seen upstream of *MMP9* and are separated from *MMP9* by a recombination hotspot. Closer to the strongest association signal are *ZNF335* and *PCIF1*. There is no literature evidence for any potential link for ZNF335 to AAA and the only identified genetic association of *ZNF335* is with celiac disease.[42] Although rs181914932 is upstream and more proximal to PCIF1, it has been associated with the activity of PLTP,[43] an adjacent gene in the same locus. Our eQTL analyses demonstrated an association between the lead SNP we assessed at this locus (rs3827066) and PLTP expression in aortic tissue (Online Tables XX and XXI). We have also shown that PLTP expression is significantly higher in aneurysmal aortic

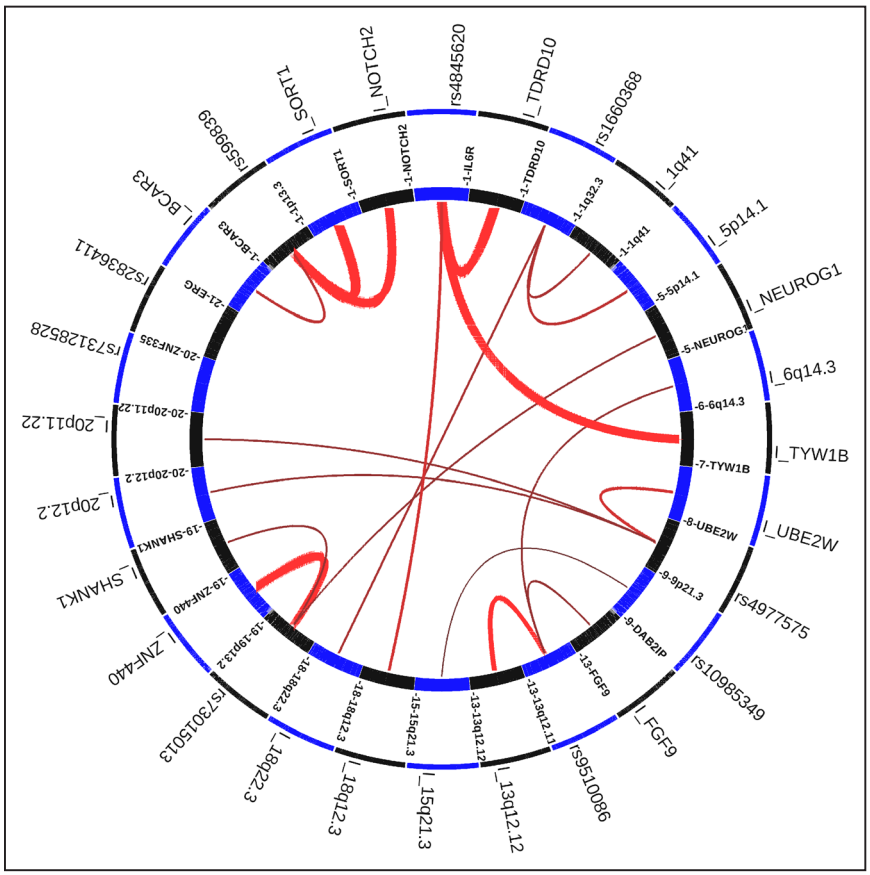

Figure 4. Circle plot showing the lead single nucleotide polymorphism (SNP) distal interaction regions based on the 9 replicated abdominal aortic aneurysm genome-wide association study SNPs. Top variants with highest regulatory signals and distal interaction regions are shown on the outer circle (significant regulatory variants are labeled with l). The inner circle shows genes and genomic loci, whereas the distal interactive signals are shown with red lines (width corresponds to intensity of interaction). Note the long-range interactions, such as that between variants associated with *IL6R* (rs4845620, 1q21.3) and *TYW1B* (7q11.23).

tissue than in control aorta (Online Table XXIII and Figure IV). PLTP plays a role in cholesterol transport. These data strengthen the evidence, particularly when taken together with the *SORT1* and *LDLR* associations confirmed here, that aberrations of lipid metabolism play a key role in the development of AAA.

The other novel AAA loci identified here contain *LINC00540*, *ERG*, and *SMYD2*. *LINC00540* is a long noncoding RNA with no currently known function; however, both our GWAS3D and eQTL analyses independently suggested an association with FGF9, which was also differentially expressed within AAA tissue. *ERG* encodes a TF that is normally present in hematopoietic and endothelial cells. ERG has a role in vascular endothelial growth factor/mitogen-activated kinase-like protein–mediated vascular development,[44] as well as regulating angiogenesis, which is known to play a role in the development of AAA.[45,46] ERG also plays a role in the embryonic development of the aorta,[44] and it has been hypothesized that in utero aortic development has a role in the later development of an AAA.[47] In prostate cancer, ERG has been shown to regulate the expression of MMP9.[48] Taken together this limited evidence points to several potential roles by which ERG may influence the development of AAA and, along with our significant eQTL observations, strongly suggest that further work in this area is warranted.

The role of SMYD2 in AAA is less clear. SMYD2 regulates *HSP90* (heat shock protein 90) methylation,[49] and the inhibition of heat shock protein 90 has been shown to reduce AAA formation in murine models,[50] suggesting this as a possible link between SMYD2 and AAA. SMYD2 also plays a role in the differentiation of embryonic stem cells,[51] again suggesting a possible role for aberrations of in utero aortic development influencing the risk of aortic disease later in life.

The integrated gene function analysis tool DEPICT identified numerous pathways that are potentially relevant to aneurysm pathogenesis (Table 2). In particular, we note with interest that the strongest predicted set was associated with long bone epiphyseal plate formation, which is possibly consistent with previous studies reporting tall stature as a risk factor for AAA[52] and conversely short stature with occlusive CAD.[53,54]

Our network analyses using 2 different bioinformatics tools also revealed a central role for MMP9 in AAA, with IPA identifying direct interactions between ERG, IL6R and LDLR, and MMP9, and Consensus PathDB identifying a direct interaction between ERG and MMP9 with secondary interactions between both SMYD2 and LDLR and MMP9. These results suggest that the novel loci could act in concert, either synergistically or antagonistically, to affect the AAA phenotype, and provide hypotheses for future investigation using animal and cell culture models.

In this study, we did not replicate the association previously identified between *LRP1* and AAA.[7] The samples from the original study that identified this association were included in this analysis, suggesting that this may have been a false-positive association. However, there is evidence supporting LRP1 as a biologically plausible candidate pathway for AAA.[55,56] Variants at, or close to, LRP1 are also associated with other vascular/related phenotypes (aortic dissection,[57] migraine,[58] and lipid traits[17]). Because we observed a degree

Table 2. DEPICT Gene Enrichment Sets Based on the Top 10 Validated Loci

Original Gene Set ID	Original Gene Set Description	DEPICT Nominal *P* Value
MP:0006396*	Decreased long bone epiphyseal plate size	1.14×10^{-9}
GO:0034381	Plasma lipoprotein particle clearance	5.22×10^{-7}
ENSG00000132005	RFX1 PPI subnetwork	2.28×10^{-6}
MP:0005595	Abnormal vascular smooth muscle physiology	1.55×10^{-3}
ENSG00000122641	INHBA PPI subnetwork	1.79×10^{-3}
MP:0002764	Short tibia	1.79×10^{-3}
ENSG00000169047	IRS1 PPI subnetwork	2.21×10^{-3}
GO:0050431	Transforming growth factor beta binding	2.47×10^{-3}
MP:0005590	Increased vasodilation	3.45×10^{-3}
GO:0071813	Lipoprotein particle binding	3.51×10^{-3}
GO:0005178	Integrin binding	4.08×10^{-3}
ENSG00000133056	PIK3C2B PPI subnetwork	4.40×10^{-3}
MP:0005095	Decreased T cell proliferation	4.84×10^{-3}
ENSG00000149257	SERPINH1 PPI subnetwork	5.79×10^{-3}
ENSG00000034152	MAP2K3 PPI subnetwork	6.58×10^{-3}
ENSG00000017427	IGF1 PPI subnetwork	7.51×10^{-3}
GO:0043406	Positive regulation of MAP kinase activity	7.65×10^{-3}
MP:0000180	Abnormal circulating cholesterol level	7.71×10^{-3}
MP:0001915	Intracranial hemorrhage	8.00×10^{-3}
MP:0004883	Abnormal vascular wound healing	8.15×10^{-3}
ENSG00000106992	AK1 PPI subnetwork	8.69×10^{-3}
MP:0003419	Delayed endochondral bone ossification	8.98×10^{-3}
MP:0000716	Abnormal immune system cell morphology	9.63×10^{-3}
ENSG00000170581	STAT2 PPI subnetwork	9.79×10^{-3}
GO:0043277	Apoptotic cell clearance	9.98×10^{-3}
MP:0001828	Abnormal T—ell activation	0.01
ENSG00000141506	PIK3R5 PPI subnetwork	0.01
GO:0000989	Transcription factor binding/transcription factor activity	0.01
GO:0007254	JNK cascade	0.01
GO:0014910	Regulation of smooth muscle cell migration	0.02
MP:0001552	Increased circulating triglyceride level	0.02
MP:0001559	Hyperglycemia	0.02
ENSG00000105851	PIK3CG PPI subnetwork	0.02
GO:0050900	Leukocyte migration	0.03
MP:0003957	Abnormal nitric oxide homeostasis	0.03
GO:0006953	Acute-phase response	0.03
ENSG00000206240	HLA-DRB1 PPI subnetwork	0.03
GO:0043123	Positive regulation of I-kappaB kinase/NF-kappaB cascade	0.03
ENSG00000145431	PDGFC PPI subnetwork	0.04
MP:0008706	Decreased interleukin-6 secretion	0.04
MP:0008688	Decreased interleukin-2 secretion	0.04

(*Continued*)

Table 2. Continued

This table is a truncated version of the full list available in the Online Table XVIII. DEPICT indicates Data-Driven Expression-Prioritized Integration for Complex Traits.

*The gene sets that had a false discovery rate of <0.2.

of heterogeneity at this locus in our analysis (Online Table VII), we consider that further investigation of this locus remains warranted despite our findings.

Our GWAS3D genome analysis predicted potential novel biological pathways in AAA pathogenesis. For example, *FGF9* was shown to have a possible distal interaction with the intergenic SNP rs9316871. FGF9, although not previously considered a strong candidate in AAA pathogenesis, was nevertheless at least partially validated by its increased mRNA expression in AAA tissue (Table 3). In AAA, both the medial and adventitial layers of the vessel wall are significantly more vascularized compared with nonaneurysmal tissue,[59] and it is therefore interesting to note that FGF9 has been shown to enhance angiogenesis and neovascularization within mouse models of myocardial infarction.[60]

The main strength of this study is the inclusion of all currently available worldwide GWAS data sets for AAA and formation of an expanded International Aneurysm Consortium. We acknowledge several limitations in our work. The overall numbers of samples included in our analysis are lower than for more common traits such as diabetes mellitus[15] and CAD.[16] We also did not have an adequate number of females in our sample set to perform sex-specific analyses that may

Table 3. Genes Predicted by GWAS3D Analysis to Be Associated With Putative AAA Loci Identified in the Discovery Study Demonstrating Significantly Different mRNA Expression in Aneurysmal Aortic Wall Samples From 49 Patients With AAA Compared With 10 Organ Donor Control Aortic Samples

GWAS3D Gene Selection Criteria	Gene	Locus	mRNA *P* Value	AAA mRNA expression
Predicted distal interaction (SORT1)	BCAR3	1p22.1	1.8×10^{-4}	Decreased
SNP in proximity with lead SNP at AAA locus	SORT1	1p13.3	1.1×10^{-4}	Decreased
Predicted distal interaction (SORT1)	NOTCH2	1p12	4.6×10^{-7}	Increased
Predicted distal interaction (IL6R)	TDRD10	1q21.3	0.006	Increased
Predicted distal interaction (CDKN2BAS1/ANRIL)	UBE2W	8q21.11	0.030	Increased
SNP in proximity with lead SNP at AAA locus	CDKN2BAS1/ ANRIL	9p21.3	0.003	Increased
SNP in proximity with lead SNP at AAA locus	LRP1	12q13.3	0.008	Decreased
SNP in proximity with lead SNP at AAA locus	NAB2	12q13.3	1.1×10^{-5}	Decreased
Predicted distal interaction (LINC0540)	FGF9	13q11	0.002	Increased
SNP in proximity with lead SNP at AAA locus	PLTP	20q13.12	0.011	Increased

Nonsignificant results (30 genes) are shown in Online Table XXIII, and box and whiskers plots on mRNA expression levels are presented in Online Figure IV. See Figure 4 for results from the GWAS3D analysis. AAA indicates abdominal aortic aneurysm; GWAS, genome-wide association studies; and SNP, single nucleotide polymorphism.

have been informative given the strong sexual dimorphism exhibited by AAA.[61] We recognize this limitation, but the current focus of AAA screening programs on men alone[2,3] and the much reduced prevalence of AAA in women means that collecting adequate samples for such analyses is likely to be challenging. Some of the contributing GWAS studies such as the Aneurysm Consortium GWAS were derived from multicenter sample collections that led to intercohort heterogeneity in clinical phenotyping of the case groups. Together with the limited covariate data available for the control groups in the GWAS studies that used population control samples, this led to an inability to reliably adjust for clinical covariates in our overall analysis. Given these limitations, and in particular about the numbers of samples available for analysis in AAA, alternative approaches for investigating the genetic cause of AAA need to be considered. The natural history of AAA with a long latent period (if detected early), during which patients are monitored by serial imaging studies, offers the opportunity to study disease progression as a continuous trait, leveraging additional power over discrete trait approaches for the limited sample sizes available.[37]

In conclusion, our meta-GWAS and the bioinformatics analyses, applying multiple techniques, has highlighted several potentially novel mechanisms of AAA pathobiology. These will require direct investigation in future studies to confirm their role in the development and progression of AAA.

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Appendix

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